Fig. S1

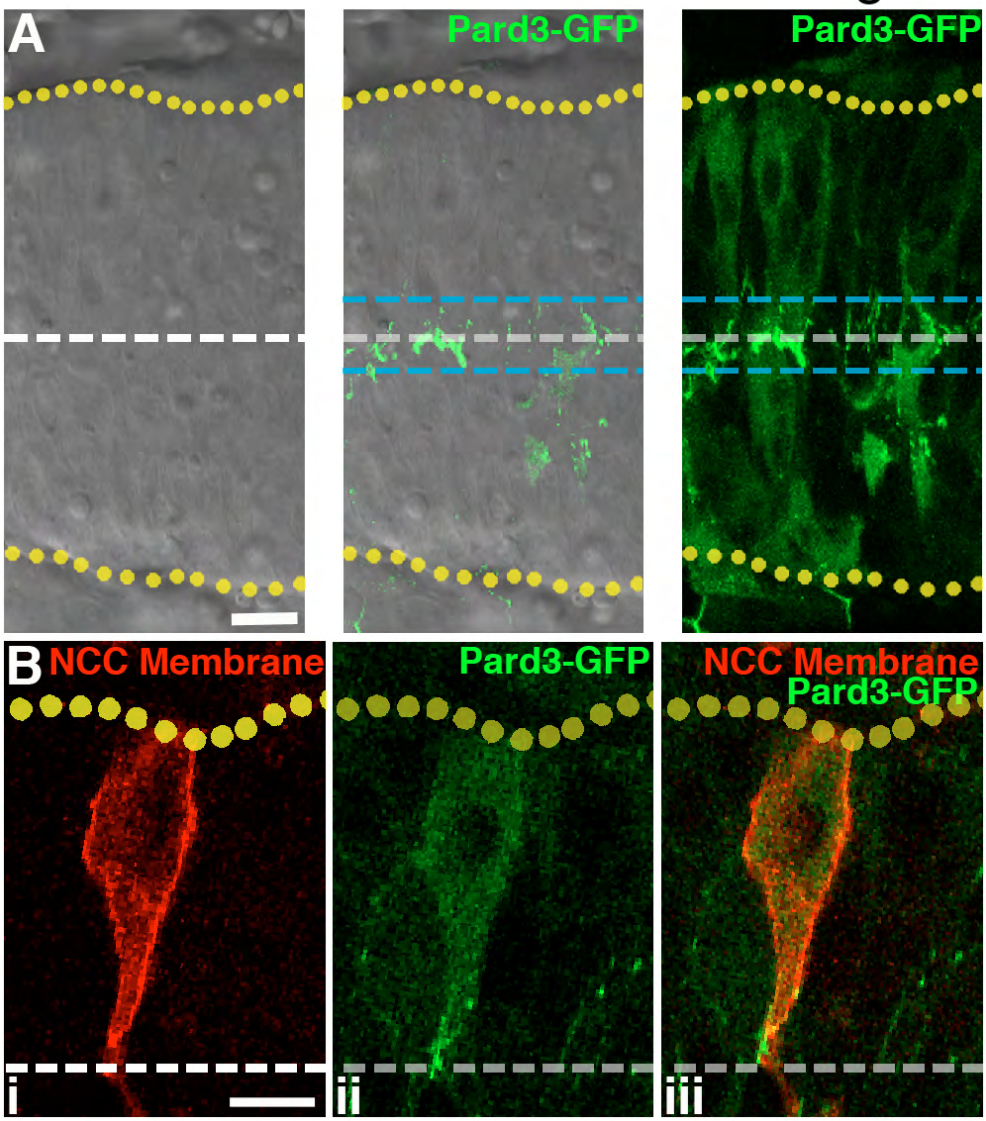


Fig. S1. Premigratory NCC tails, identified by the embryonic midline, express the apical marker Pard3-GFP. (**A**,**B**) Confocal images of single timepoints in live embryos (dorsal views, anterior left). Yellow dotted lines mark basal surfaces of the neuroepithelium and white dashed lines mark apical midlines, identified by DIC. (A) Single plane of DIC (i, ii) in an embryo broadly expressing Pard3-GFP (*z*-projection, ii, iii). The blue dashed lines (ii, iii) are 5 μm from the midline and the majority of Pard3-GFP is observed in this area. (B) Single plane of NCCs expressing mCherry-CAAX (i, iii) and Pard3-GFP (ii, iii). The NCC was identified as premigratory (tail at the midline) and the tail showed the apical marker Pard3-GFP. Scale bars: 10 μm.

Fig. S2

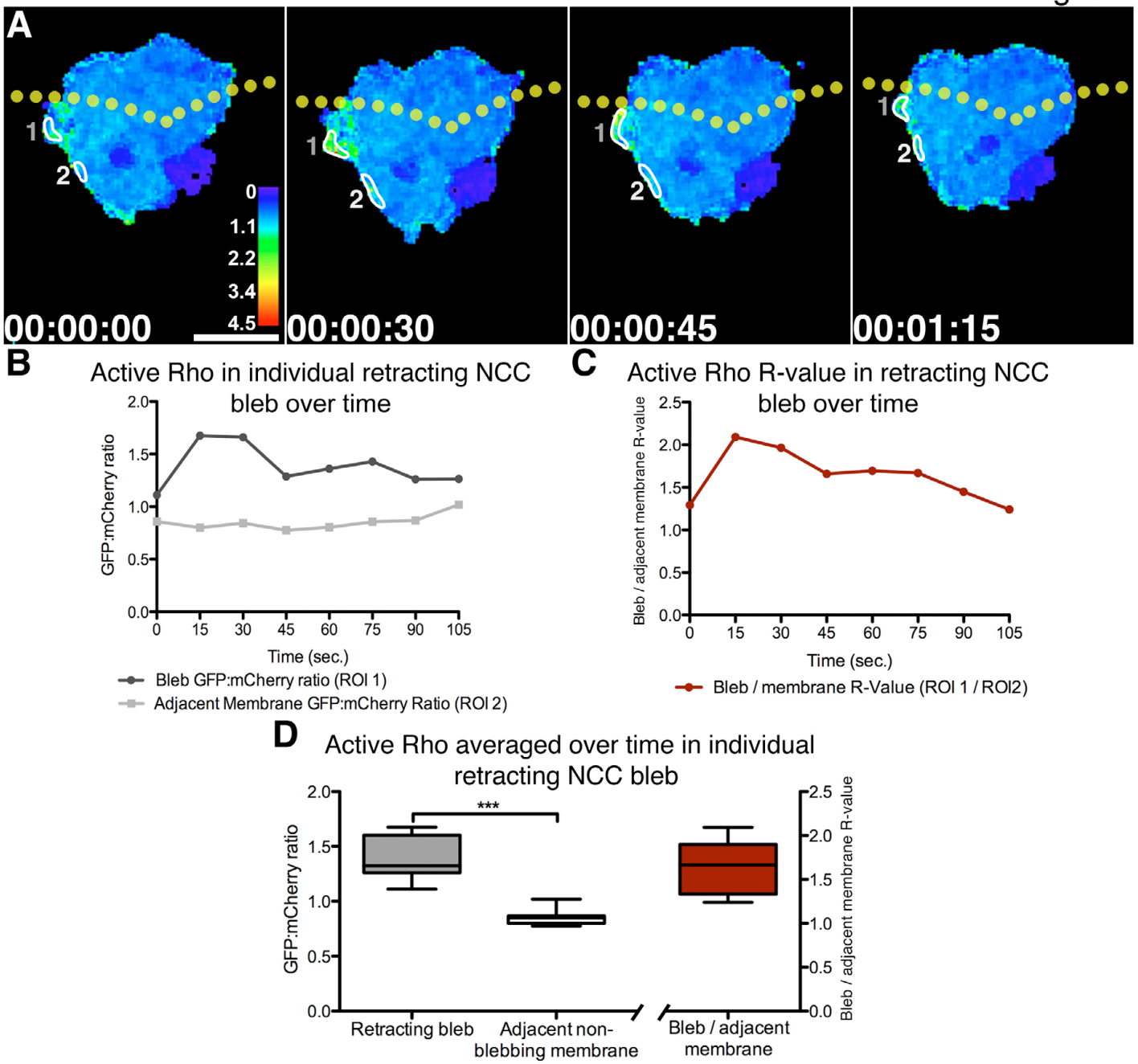


Fig. S2. Calculation of R-values in NCC bleb. (**A**) Time-lapse images (dorsal views, anterior left, confocal z-projections) of NCC labeled with GFPrGBD and mCherry. ROIs were used to calculate GFP/mCherry intensity in blebbing (1) and non-blebbing (2) membrane. Look-up table shows GFP/mCherry intensity. Yellow dotted lines mark the basal neuroepithelium. (**B**) GFP/mCherry intensity in bleb (dark line) and non-blebbing membrane (light line) over time beginning at time of maximum bleb extension for cell shown in A. (**C**) Active Rho R-values (ROI1/ROI2) plotted over time. (**D**) Average GFP/mCherry intensity over time is significantly higher in blebbing than non-blebbing membrane (left axis, ***P<0.001, paired one-tailed, t-test, see Table S1 for all blebs). Average of active Rho R-values (right axis) is 1.62 (62% higher active Rho in bleb). Scale bar: 10 μm.

Fig. S3

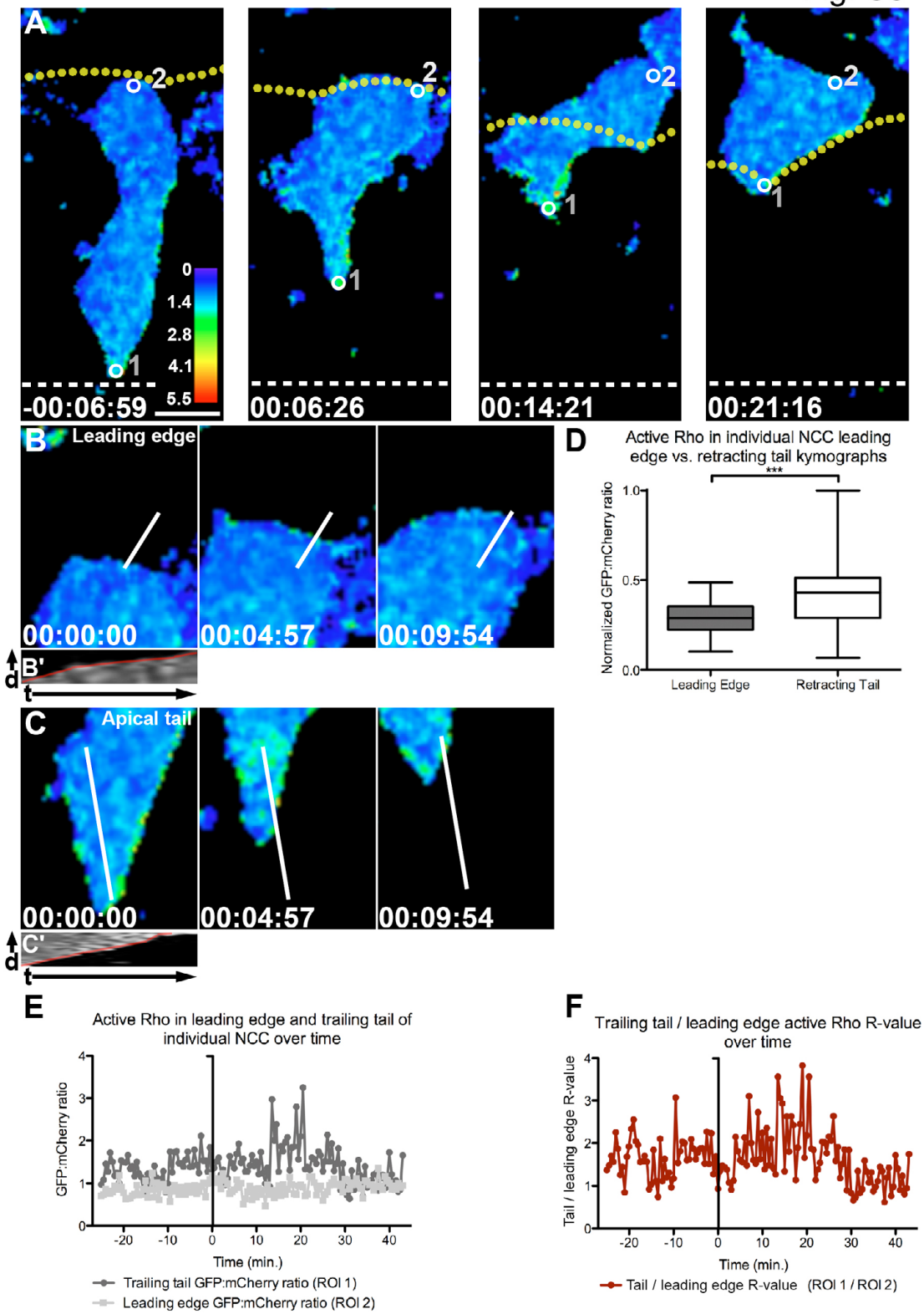


Fig. S3. Calculation of R-values in NCC tail. (**A**) Time-lapse images (dorsal views, anterior left, confocal z-projections) of NCCs labeled with GFPrGBD and mCherry. Circles show ROIs used to calculate GFP/mCherry intensity in NCC tail (1) and leading edge (2). Look-up table shows GFP:mCherry ratio intensity. Yellow dotted lines mark the basal surface of the neuroepithelium and white dashed lines mark the apical midline. In A,B,C,E,F, apical detachment is 0 minutes. (**B**,**C**) Higher magnification views of leading edge and tail, showing lines used for kymographs. (**B′**,**C′**) Kymographs of normalized GFP/mCherry intensity over time. The red line represents the border of the cell on the kymograph. GFP/mCherry intensity was measured along this line and averaged to determine the active Rho level. (**D**) Average normalized GFP/mCherry intensity in tail (from C′) was significantly higher than the leading edge (from B′; ***P<0.001, paired one-tailed, t-test, see Table S2 for all tail retraction events). (**E**) GFP/mCherry intensity for the tail (dark line) and leading edge (light line) over time for individual NCCs. (**F**) Active Rho R-value (ROI1/ROI2) plotted over time. Scale bar: 10 μm.

Fig. S4

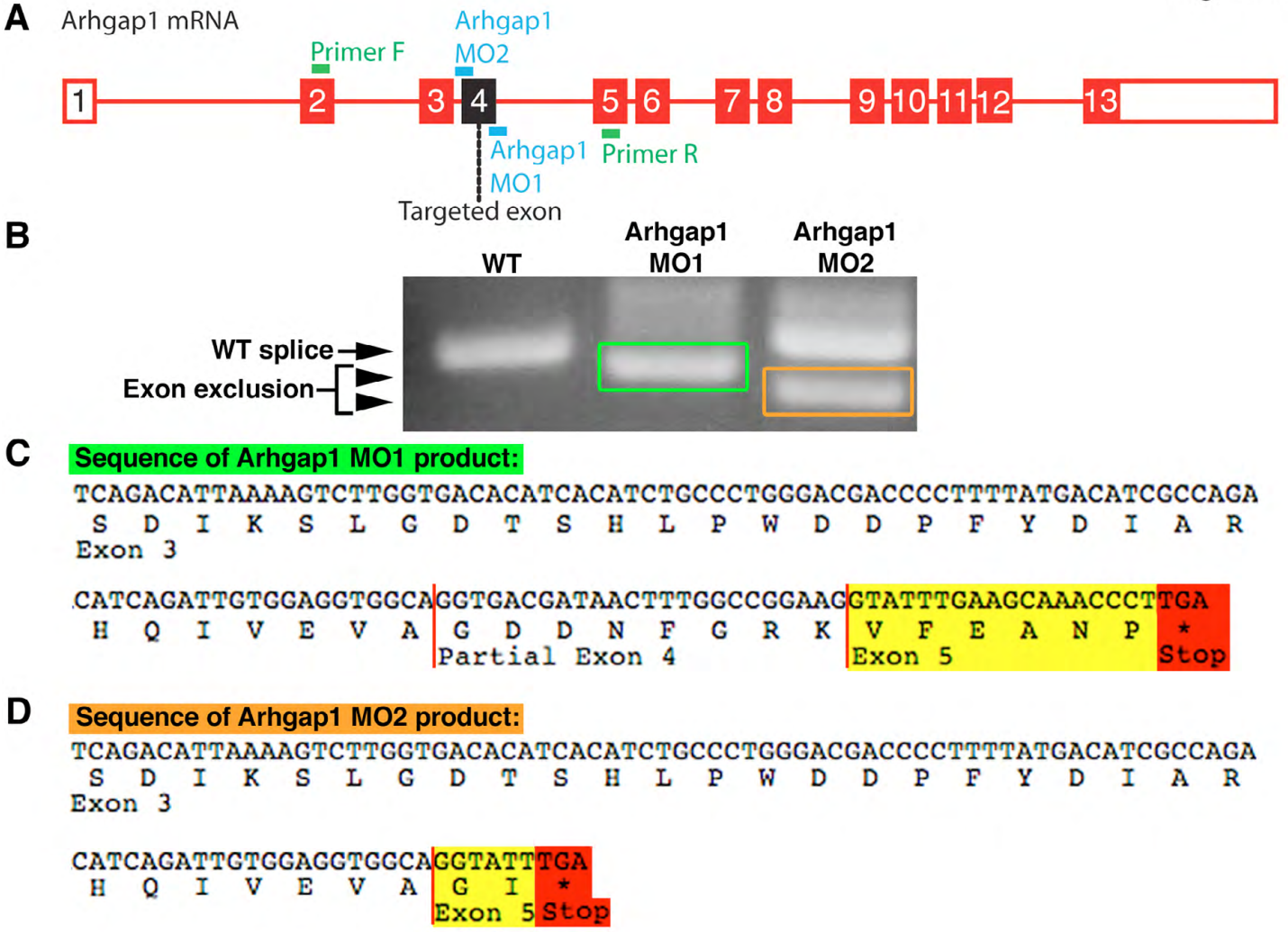
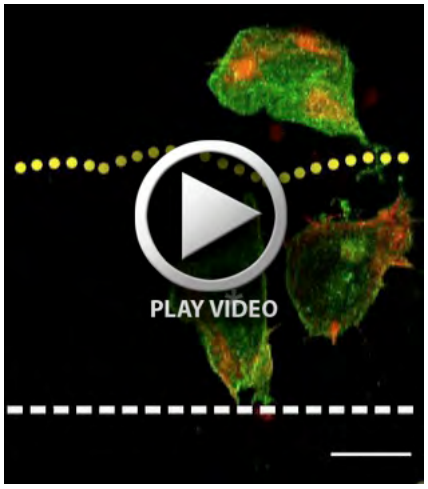
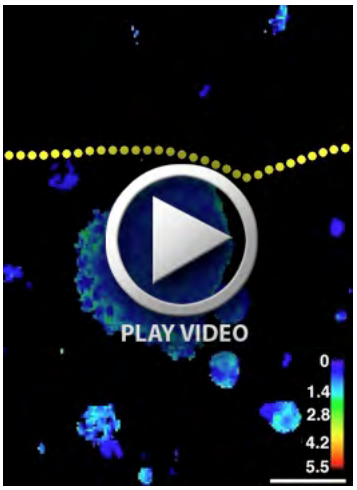


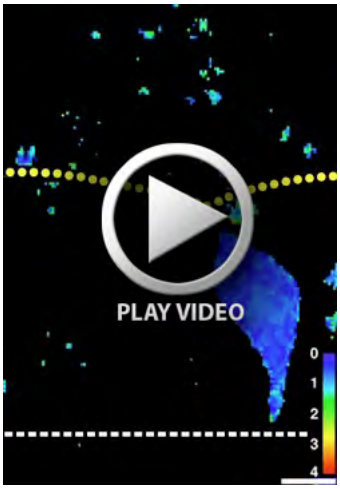
Fig. S4. Morpholinos against Arhgap1 disrupt normal splicing. (**A**) Schematic of zebrafish Arhgap1. Exons are numbered and open boxes represent untranslated regions. Morpholino target sites and primers for PCR from cDNA are shown. (**B**) Arhgap1 PCR products using cDNA from uninjected (wild type), Arhgap1 MO-1- and Arhgap1 MO-2-injected embryos. Both morpholinos result in smaller bands, which correspond to exon exclusion. MO-2 also showed a band corresponding to wild-type Arhgap1 splicing. (C,D) Sequence of PCR products resulting from morpholino injections. (**C**) Arhgap1 MO-1 injection resulted in exclusion of most of exon 4; however, it appears a cryptic splice site was found. The missplicing resulted in a frame shift (highlighted in yellow) and a premature stop (red). (**D**) Arhgap1 MO-2 injection resulted in complete exclusion of exon 4 and introduced a frame shift (yellow) and premature stop (red).



Movie 1. F-Actin transiently accumulates in NCC tails prior to detachment. Movie of NCCs labeled with GFP-CAAX and mCherry-UtrCH. Images were acquired every 30 seconds; movie shows 63 minutes. In the first frame, the cell marked with the asterisk is in a premigratory morphology; the yellow dotted line marks the basal surface of the neuroepithelium; the white dashed line marks the midline. Scale bar: 10 μm.



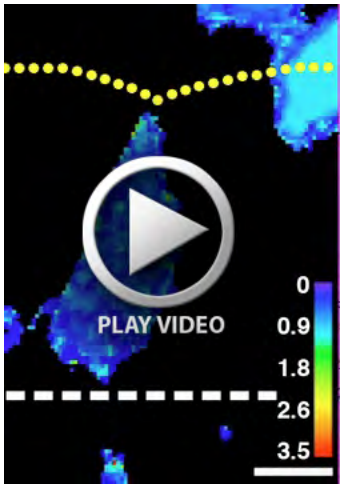
Movie 2. Rho is activated in retracting NCC blebs. Movie of NCCs labeled with GFPrGBD and mCherry. The ratio channel of GFP:mCherry is shown. Images were captured every 10 seconds; movie shows 4 minutes. In the first frame, the yellow dotted line marks the basal surface of the neuroepithelium; the look-up table shows the GFP:mCherry ratio. Scale bar: 10 μm.



Movie 3. Rho is activated in apical tails prior to detachment and during tail retraction. Movie of NCCs labeled with GFPrGBD and mCherry. The ratio channel of GFP:mCherry is shown. Images were acquired every 30 seconds; movie shows 75 minutes. In the first frame, the yellow dotted line marks the basal surface of the neuroepithelium; the white dashed line marks the midline; the look-up table shows the GFP:mCherry ratio. Scale bar: 10 μm.

Movie 4. Apical NCC tail detachment is disrupted during C3 treatment. Movie of NCC labeled with GFP-CAAX treated with C3. Images were acquired every 30 seconds; movie shows 123 minutes. In the first frame, the yellow dotted line marks the basal surface of the neuroepithelium; the white dashed line marks the midline. Scale bar: 10 μm.

Movie 5. Apical tail detachment is disrupted during ROCKout treatment. Movie of NCCs labeled with GFP-CAAX treated with ROCKout. Images were acquired every 30 seconds; movie shows 171 minutes. In the first frame, the yellow dotted lines mark the basal surface of the neuroepithelium; the white dashed line marks the midline. Scale bar: 10 μm.



Movie 6. Arhgap1 knockdown leads to expanded Rho activation. Movie of NCCs expressing GFPrGBD and mCherry in embryos injected with Arhgap1 morpholino. Images were acquired every 30 seconds; movie shows 26 minutes. In the first frame, the yellow dotted lines mark the basal surface of the neuroepithelium; the white dashed line marks the midline; the look-up table shows the GFP:mCherry ratio. Scale bar: 10 μm.

Table S1. Comparison of active Rho GFP/mCherry intensity averaged over time in retracting blebs versus adjacent non-blebbing membrane

	GFP/mCherry intensity averaged over time				
Bleb number	Bleb ratio (mean)	s.d.	Adjacent membrane ratio (mean)	s.d.	*P* value (paired *t*-test, bleb versus membrane)
1	0.99	0.17	0.54	0.18	0.0387
2	1.04	0.10	0.83	0.03	0.0044
3	1.21	0.12	0.68	0.09	0.0006
4	0.79	0.12	0.66	0.04	0.1413
5	0.96	0.08	0.80	0.05	0.014
6	0.92	0.06	0.71	0.05	0.0122
7	1.09	0.22	0.87	0.04	0.087
8	1.53	0.13	0.96	0.09	<0.0001
9	1.66	0.19	0.82	0.04	0.0004
10	1.56	0.18	0.96	0.07	0.0025
11	1.82	0.49	1.05	0.13	0.006
12	0.97	0.07	0.74	0.06	0.0061
13	1.01	0.11	0.84	0.03	0.0493
14	1.38	0.20	0.87	0.11	0.0002
15	1.31	0.25	0.85	0.06	0.0304

Table S2. Comparison of active Rho over time averaged in leading edge versus trailing tail as measured from kymographs

Retraction event	Normalized GFP/mCherry intensity over time				
	Leading edge	s.d.	Trailing tail	s.d.	*P* value (paired *t*-test, leading versus trailing edge)
1	0.30	0.17	0.52	0.13	<0.0001
2*	0.29	0.08	0.41	0.16	<0.0001
3*	0.43	0.11	0.61	0.14	<0.0001
4*	0.41	0.08	0.52	0.27	<0.0001
5	0.60	0.12	0.66	0.20	<0.0001
6	0.24	0.06	0.32	0.19	<0.0001
7	0.40	0.04	0.50	0.17	<0.0001
8*	0.26	0.08	0.58	0.13	< 0.0001
9	0.37	0.05	0.47	0.17	<0.0001
10	0.37	0.09	0.70	0.14	<0.0001

*Indicates apical detachment